

# COMPOSITIONS AND METHODS FOR USE AGAINST ACNE-INDUCED INFLAMMATION AND DERMAL MATRIX-DEGRADING ENZYMES

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## TECHNICAL FIELD

This invention involves protecting human skin from some of the effects of acne, especially *acne vulgaris*, through the use of the topically and/or systemically applied non-retinoid and non-steroid compounds that diminish inflammation and matrix-degrading enzymes in acne-affected skin.

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## BACKGROUND

Acne is a multifactorial disease, developing in the sebaceous follicles. At least one agent thought responsible is the anaerobe *Propionibacterium acnes* (*P. acnes*); in younger individuals, practically no *P. acnes* is found in the follicles of those without acne.

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The disease of acne is characterized by a great variety of clinical lesions. Although one type of lesion may be predominant (typically the comedo), close observation usually reveals the presence of several types of lesions (comedones, pustules, papules, and/or nodules). The lesions can be either noninflammatory or, more typically, inflammatory. In addition to lesions, patients may have, as the result of lesions, scars of varying size. The fully developed, open comedo (*i.e.*, a plug of dried sebum in a skin pore) is not usually the site of inflammatory changes, unless it is traumatized by the patient. The developing microcomedo and the closed comedo are the major sites for the development of inflammatory lesions. Because the skin is always trying to repair itself, sheaths of cells will grow out from the epidermis (forming appendageal structures) in an attempt to encapsulate the inflammatory reaction. This encapsulation is often incomplete and further rupture of the lesion typically occurs, leading to multichanneled tracts as can be seen in many acne scars.

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In general, there are four major principles presently governing the therapy of acne: (i) correction of the altered pattern of follicular keratinization; (ii) decrease sebaceous gland activity; (iii) decrease the follicular bacterial population (especially *P. acnes*) and inhibit the production of extracellular inflammatory products through

the inhibition of these microorganisms; and (iv) produce an anti-inflammatory effect. The present treatments for acne following these principals typically include: vitamin A acid (retinoic acid), known for its comedolytic properties, administered topically (e.g., Retin-A® brand 0.025% all-*trans* retinoic acid cream) or systemically (e.g., Accutane® brand 13-*cis* retinoic acid); an antibiotic administered systemically (e.g., tetracycline or one of its derivatives) or topically (e.g., benzoyl peroxide, erythromycin, clindamycin, azelaic acid); the use of other comedolytic agents such as salicylic acid; or the use of systemic anti-androgens such as cyproterone acetate and spironolactone (because androgens promote sebum production, and sebum has been found to be comedogenic and inflammatory), which may be administered in combination with an estrogen. Atrophy, the most feared side effect of topical glucocorticoids, is seen as an overall reduction in the dermal volume and occurs as early as one week after superpotent-steroid use. Systemic side effects of chronic glucocorticoid use include suppression of the hypothalamic-pituitary-adrenal (HPA) axis, Cushing's syndrome, glaucoma, and, in children, failure to thrive. (Children, especially infants and young children, are at higher risk for systemic side effects due to their greater surface-to-body ratio. They also may not metabolize corticosteroids as well as adults.) Withdrawal symptoms can appear after topical steroids have been used for a long period of time. Severe flaring may occur when isotretinoin (13-*cis*) therapy is started, and so concomitant use of a steroid, and suboptimal doses of isotretinoin, are often required at the start of therapy; additionally, retinoids generally are teratogenic (inhibiting organogenesis as opposed to being mutagenic).

The art has addressed inflammation and scarring caused by acne as a secondary benefit to the treatment of the disease; that is, if the acne is cured the factors causing scarring will be eliminated. There is otherwise no treatment directed at preventing scarring from acne. Neither is there presently any direct treatment for the inflammation accompanying acne. The conventional treatment acts to prevent further problems by alleviating the cause of the acne; for example, a patient is treated with tetracycline, an antibiotic, in hopes of killing the *P. acnes*, and the death of the bacteria will effectively end the inflammation and future scarring. Much as antipyretics, analgesics, decongestants, and antihistamines have been developed to treat the symptoms of colds and upper respiratory infections (as opposed to

antibiotics and antivirals to kill off the invading bacteria and viruses), there is a need for treatments diminishing if not preventing scarring and inflammation in acne.

## SUMMARY OF THE INVENTION

One object of this invention is to reduce and/or eliminate scarring in acne-affected skin.

Another object of this invention is to reduce and/or eliminate the inflammatory reaction that accompanies acne.

We have discovered that acne lesions are not only inflammatory, but that enzymes that degrade the dermal matrix, including metalloproteinases (MMPs) and other proteases such as neutrophil elastase, are present in the lesion, and these enzymes likely cause, or at least significantly contribute, to scarring. Additionally, the *P. acnes* products from the lesion are believed to initiate a signalling cascade which both induces these degradatory enzymes within the skin and also induces PMNs (polymorphic leukocytes; e.g., neutrophils) to migrate to the lesion and contribute to the induction of degradatory enzymes. Still further, the *P. acnes* products are believed to cause a signalling cascade leading to inflammation.

Our invention is the treatment and prevention of scarring caused by acne, which treatment and prevention is accomplished through the administration of a composition comprising an effective amount of an non-retinoid, non-glucocorticoid inhibitor of a dermal matrix-degrading enzyme to acne-affected skin. Administration may be topical, systemic (preferably oral), or a combination thereof, and may be given in combination with another, conventional acne therapy (e.g., benzoyl peroxide, a retinoid, or a tetracycline). In yet another embodiment, the topical application of a dermal matrix-degrading enzyme inhibitor includes the administration of inhibitors of both MMPs and other proteases. The inhibitor can be a direct inhibitor, acting specifically on the enzyme, or an indirect inhibitor, tying up a signalling compound in a pathway leading to the matrix-degrading enzyme.

Another aspect of the invention is the administration of a compound that inhibits the inflammatory reaction and/or the recruitment of cells resulting in an inflammatory reaction in the acne lesion. Likewise, this administration can be topical, systemic (e.g., oral), or a combination thereof. Similarly, this administration can be accompanied by the co-administration of a conventional acne therapy.

## BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts Western analysis of MMP-8 in human skin *in vivo* affected with an acne lesion, in uninvolved skin adjacent to the lesion.

Figs. 2A and 2B are photomicrographs of a skin biopsy of acne-affected skin and uninvolved skin from the same human volunteer, where the biopsies are stained to reveal the presence of neutrophil elastase.

Figs. 3A and 3B are photomicrographs of a skin biopsy of acne-affected skin and uninvolved skin from the same human volunteer, where the biopsies are stained to reveal the presence of MMP-1, Figs. 3A and 3B being taken at a deeper level of the dermis.

Figs. 4A and 4B are color photomicrographs of a skin biopsy of uninvolved (4A) and acne-affected skin (4B), each having been stained to reveal the presence of Type I procollagen.

Fig. 5A is a photomicrograph of a skin biopsy of an acne lesion stained to reveal the presence of neutrophil elastase, and Fig. 5B is an *in-situ* zymogram showing neutrophil collagenase activity in an acne lesion.

Fig. 6 is a cartoon depicting a possible mechanism for the present invention.

Figs. 7A-7F are photomicrographs of zymograms of skin biopsies showing the presence (or absence) of collagenase activity in uninvolved skin (7A), untreated acne-affected skin (7B), control-treated acne-affected skin (7C), and in treated acne-affected skin (7D-7F).

Fig. 8A and 8B are graphical representations of data obtained from biopsies of four individuals with acne-affected skin examined for the presence of degradative enzymes and inflammatory signalling compounds.

Figs. 9A and 9B are immunohistology photomicrographs of fluorescently-labelled p65 antibody specific for NF- $\kappa$ B, Fig. 9A from uninvolved skin showing the staining in the body of the cells, and Fig. 9B from an acne lesion showing the staining in the nucleus of the cell.

## DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The matrix of the skin (the dermal matrix), a structural framework that supports the cells and other structures in the skin, is comprised of collagen and elastin proteins for structural and dynamic (elastic) support.

Scarring of acne-affected skin has been known for a long time, and the typical treatment philosophy is that curing the acne will eliminate future scarring. As described in the Background section, it has also been known that acne includes bacterial infestation and an inflammatory reaction.

5 We have discovered that neutrophils (PMNs), immune cells that migrate to areas of injury, invade acne-affected skin, and release both a collagenase (MMP-8) and another protease (neutrophil elastase) that likely exacerbate scarring. Additionally, we have discovered that acne-affected skin has an elevated collagenase (MMP-1) level from resident skin cells that further exacerbates scarring. 10 By inhibiting these dermal matrix-degrading enzymes, scarring of acne-affected skin can be lessened. Neutrophils circulate in the blood and therefore must be recruited by a signalling mechanism to induce their presence in the skin, facilitate their infiltration to the affected site, and enable their release of MMP-8 and elastase. Accordingly, impeding or disrupting the signalling which induces their presence in the skin and/or the activity of MMP-8 or elastase is likely to diminish the 15 accompanying inflammation and the degradatory action of MMP-8 and/or elastase.

Matrix metalloproteinases (MMPs) are a family of enzymes that play a major role in physiological and pathological destruction of connective tissue, especially collagen. Various types of collagen and collagenases (types of MMPs) are known in this field, and a further description can be found in US 5,837,224, and in our co- 20 pending application 89,914, filed 3 June 1998, the disclosures of which are incorporated herein by reference in their entirety for all purposes. Inhibitors of MMPs (e.g., direct inhibitors of the proteinase) and of molecular pathways (e.g., inhibitors of AP-1 and/or NF- $\kappa$ B) that affect MMP expression are known in other fields and likewise are described in US 5,837,224 and the 89,914 co-pending application. 25

MMP-8 preferentially degrades Type I collagen and is a more active enzyme than MMP-1 and so degrades collagen better. Neutrophils also release an elastase, a serine protease, an enzyme that degrades elastin protein in the dermal matrix.

This invention inhibits scar formation in acne-affected skin by inhibiting MMPs, especially neutrophil collagenase, generated in such acne-affected skin, and by 30 inhibiting other dermal matrix-degrading enzymes. This invention also inhibits the redness (erythema) and discomfort caused by the inflammatory reaction accompanying acne.

For the experiments, the results of which are shown in the figures described below, human volunteers (each having given informed consent) were used to assess the differences in collagenase and elastase concentration between areas of the skin having an acne lesion and uninvolved adjacent skin areas, and to evaluate *ex vivo* the likely effectiveness of certain inhibitors.

Fig. 1 depicts a Western blot of MMP-8 collagenase protein in human skin biopsied from an area having an acne lesion (L in the figure) and from clear, uninvolved, adjacent areas of skin (C in the figure) in patients ("PT") 1, 2, and 3. As shown in this figure, all three patients clearly have MMP-8 present in the acne lesion, and have no MMP-8 present in unaffected, adjacent areas of skin.

Figs. 2A and 2B are photomicrographs of stained cross-section biopsies from human dermis and epidermis of a volunteer's skin. The photographic section on the right (2B) was taken through an acne lesion, and the one on the left (2A) was taken from normal, uninvolved skin. Each biopsy section was stained to show the presence of neutrophil elastase, a matrix-degrading serine protease. The presence of neutrophil elastase in Fig. 2B indicates the presence of neutrophils in the skin, presumably due (directly and/or indirectly) to the acne. The existence of neutrophil elastase in the skin would be expected to result in a breakdown of the elastin in the dermal matrix. Thus, we have found that acne-affected skin contains a significant amount of neutrophil elastase and that uninvolved skin contains essentially no neutrophil elastase. This finding indicates that the presence of acne causes the recruitment and infiltration of immune cells to acne-affected areas of the skin.

Figs. 3A and 3B depict stained cross-sectional biopsies from a volunteer's acne-affected and uninvolved skin. The biopsies were taken and stained for the presence of collagenase (MMP-1). Figs. 3A and 3B show biopsies from acne-affected (3A) and uninvolved (3B) skin that were taken from the lower dermis of a volunteer's skin. In these two figures, stained cells are seen (3A) from acne-affected skin at the level of the dermis, whereas no staining is found in the uninvolved skin (3B) at the same level of the dermis of uninvolved (not acne-affected) skin. Thus, we have found that acne induces collagenase in the dermis through skin cells (keratinocytes, fibroblasts) resident in the skin.

Figs. 4A and 4B are photomicrographs of stained cross-sectional biopsies from the skin of a volunteer which have been stained for the presence of Type I

procollagen; Fig. 4A is from non-acne-affected skin and Fig. 4B is from acne-affected skin. (The reader is referred to our co-pending application number 285,860, filed 2 April 1999, which describes preventing UV-induced inhibition of collagen biosynthesis in human skin, and our co-pending application number 28,435, filed 24 February 1998, regarding the reduced biosynthesis of procollagen in aged skin, and so are useful for a further understanding the significance of the lack of Type I procollagen; the disclosure of those applications is incorporated herein by reference for all purposes). The presence of Type I procollagen indicates that dermal cells are producing this collagen precursor, whereby the dermal matrix is being rebuilt by new collagen. (Procollagen is made in cells and is soluble; it passes into the dermal matrix where it is formed into insoluble collagen.) Uninvolved skin, as shown in Fig. 4A, is producing procollagen as would be expected, since acne generally starts affecting people in their teens, and these people typically have skin that is producing normal, significant amounts of procollagen. However, as shown in Fig. 4B, skin from the same subject as was taken for Fig. 4A, there is almost no procollagen production in acne-involved skin. Thus, we have found that acne-involved skin is deficient in procollagen production. As we discuss in our prior applications and patents regarding photoaging, exposure of human skin to UV radiation induces in that skin MMPs, and it is believed that imperfect repair of the skin after such MMP-inducing exposure leads to dermal scarring. Based on the present results, it would appear that scarring due to acne is significantly exacerbated by the absence of procollagen in the skin: although the skin is attempting to heal itself, the lack of procollagen in that skin means that there is likely to be an imperfect repair. Thus, the more acne-involved the skin, the less procollagen production, and, combined with the existence of increased amounts of MMPs in acne-involved skin, there is a greater probability of imperfect repair of the acne lesion.

Fig. 5A depicts acne-involved skin stained for neutrophil elastase. Neutrophils enter from the blood vessel (off the picture towards the bottom) and migrate to the surface of the skin (the epidermis being shown at the top of the panel). The staining is more significant at the top of the lesion in the dermis, while there is some staining (black dots) along the bottom portion of the lesion (suggesting that there are still other neutrophils migrating to the area). The other panel (Fig. 5B) is an *in-situ* zymogram showing the presence of collagenase activity; a section of the acne lesion

is placed on a fluorescently-labelled collagen-coated slide, and where there is active collagenase that enzyme will destroy the fluorescently-labelled collagen on the slide and leave a black background on the panel. As seen in Fig. 5B, there was significant collagenase activity around the acne lesion. Thus, we have discovered that in the area of acne lesions there is neutrophil infiltration with significant elastase and collagenase activity.

While not desirous of being constrained to a particular theory, we believe that scarring due to acne is exacerbated, if not caused, by defects in skin repair. The skin is continuously trying to repair itself; in this instance from the degradation caused by acne. Acne, though more likely the bacterial infestation, leads to inflammation. The inflammatory response defense mechanism includes infiltration into the skin of neutrophil immune cells; these cells generate collagenase and elastase that degrade to dermal matrix, the degradation products being removed and the matrix then being repaired. This process of degradation, which is part of the repair process (*i.e.*, the need to breakdown and remove materials for further repair and cell growth) is not perfect, and imperfections or defects in the repaired matrix can be manifest or presented as scars. Pursuant to the present invention, the administration of an inhibitor of a dermal matrix-degrading enzyme effective to affect acne-involved skin inactivates these destructive proteins or eliminates their presence by blocking the pathway(s) that creates or activates them; whether topical or systemic, if the inhibitor is conveyed to the skin, it will be effective for inhibiting dermal matrix-degrading enzymes and thus eliminates their consequences.

Again while not desirous of being constrained to a particular theory, the possible mechanism by which this invention functions is depicted in the cartoon of Fig. 6. On the left side of Fig. 6 a hair follicle infected with *P. acnes* is shown. These bacteria release LPS (lipopolysaccharide)-like compounds which are sensed by keratinocytes (KC) (triangles in Fig. 6). (BR Vowels, S Yang, and JJ Leyden, "Induction of proinflammatory cytokines by a soluble factor of *Propionibacterium acnes*: implications for chronic inflammatory acne, *Infect. Immun.* 1995 63: 3158-3165; the disclosure of which is incorporated herein by reference). The toll-like receptor (TLR) family includes LPS receptors, and those in the keratinocytes are activated by LPS-like products from *P. acnes*. Activation of the TLRs causes NF- $\kappa$ B to enter the cell nucleus of keratinocytes, as shown in Figs. 9A and 9B discussed



below. The keratinocytes are thus induced to release chemotactic factors, especially cytokines (IL-1 $\beta$ , IL-8, IL-10, TNF $\alpha$ ). These factors activate the AP-1 and NF- $\kappa$ B pathways, and NF- $\kappa$ B activates more IL-1 and TNF $\alpha$  (a cyclical process; see Fig. 1 in our prior patent US 5,837,224 on photoaging due to UV radiation, the disclosure of which is incorporated herein by reference). The release of these factors causes inflammation, including the recruitment of neutrophils (PMNs; *i.e.*, polymorphonuclear leukocytes) from the blood supply to the acne lesion; MMP-8 and elastase are preformed in the neutrophils and so their presence in skin is due to their presence in neutrophils. As shown in this cartoon, the cytokines also effect other keratinocytes and fibroblasts (FB), which are resident in the skin, to generate MMPs. The induction of matrix-degrading enzymes due to the presence of acne, and the continual repair of the damage they do, leads to imperfect repair of the skin. Thus, elimination of the enzymes that degrade the dermal matrix reduces imperfect repair of the skin, and so lessens scarring. As shown above in Figs. 3A-3B, collagenase expression in acne-affected skin occurs in the dermis. Accordingly, a preferred composition includes indirect inhibitors of matrix degrading enzymes, such as glucocorticoids that block recruitment of neutrophils and other inflammatory immune cells, optionally retinoids that inhibit MMPs in resident skin cells, and direct inhibitors of these enzymes, such as serpine (a serine protease inhibitor analogous to TIMP), all preferably in combination with at least one compound for treating acne (*e.g.*, benzoyl peroxide or tetracycline). While retinoids and antibacterials are commonly used to treat acne, they have not been used in combination with non-retinoid MMP inhibitors, elastase inhibitors, and/or inhibitors of the PNM recruitment pathway leading to degradation of the dermal matrix.

Figs. 8A and 8B compare levels of collagen-degrading enzymes and inflammatory signalling molecules for four individuals, from their uninvolved and acne-involved skin; for the acne-involved skin, each of the volunteers is represented by a differently-shaped data point (square, circle, diamond, triangle). Fig. 8A shows the change in the amount of mRNA encoding for three different dermal matrix-degrading enzymes (MMP-1, MMP-3, and MMP-9) between uninvolved (UNINV) and acne-affected (ACNE) for these individuals; the dashed horizontal line represents the mean value; and the three different scales should be noted. In general, Fig. 8A shows that in acne-affected skin, MMP-1 is elevated an average of over 500 times

from uninvolved skin, MMP-3 is elevated an average of over 1000 times from uninvolved skin, and MMP-9 is elevated an average of almost 15 times compared with uninvolved skin. Fig. 8B shows the difference in the amount of mRNA encoding for the inflammatory cytokines mentioned above ( $\text{TNF}\alpha$ ,  $\text{IL-1}\beta$ , IL-8, and IL-10) between uninvolved and acne-affected skin; as with Fig. 8A, each of the individuals is represented by a differently-shaped data point, and the mean value is shown as the horizontal dashed line. As with the collagen-degrading enzymes, the amounts of mRNA encoding the cytokines increased from uninvolved to acne-affected skin:  $\text{TNF}\alpha$  was about four times higher,  $\text{IL-1}\beta$  was over 25 times higher, IL-8 was over 5000 times higher, and IL-10 was about 75 times higher. These data confirm that increased cytokine concentrations (inferred from an increase in their mRNA levels) are present in acne lesions, and thus the scarring and collagen degradation due to acne can be treated with a combination of MMP inhibitors and cytokine inhibitors.

Figs. 9A and 9B compare the location of fluorescent p65-labelled  $\text{NF-}\kappa\beta$  in uninvolved and acne-involved human skin biopsies via immunohistological techniques; p65 antibody forms a complex with  $\text{NF-}\kappa\beta$ , and the fluorescent labelling allows the location of the antibody to be visualized. The biopsy in Fig. 9A shows that any  $\text{NF-}\kappa\beta$  present in the epidermis is present in the body of the cells; the insert is a close-up showing that the nuclei of the cells (dark objects) are surrounded by the cell body in which the fluorescently-p65-labelled  $\text{NF-}\kappa\beta$  resides (red area). The treatment offered in this application involves, as shown in Fig. 6, activation of cells by  $\text{NF-}\kappa\beta$ . Fig. 9B, taken through an acne lesion, shows that the  $\text{NF-}\kappa\beta$  has entered the cell nucleus of keratinocytes throughout the follicular epidermis involved in the lesion, with the staining being somewhat reduced in the remaining epidermis; the insert shows that the  $\text{NF-}\kappa\beta$  has entered the cells' nuclei (hence the red fluorescence is ovoid) and that the cell body is no longer stained by the presence of  $\text{NF-}\kappa\beta$ . These figures thus show that there is prominent nuclear localization of  $\text{NF-}\kappa\beta$  in acne-involved human skin lesions *in vivo* as compared with uninvolved human skin. This nuclear localization is consistent with the mechanism proposed in Fig. 6 (but not relied upon for patentability), that  $\text{NF-}\kappa\beta$  is present in the inflammatory signalling associated with acne lesions.

In light of our findings, acne-affected patients can be helped by decreasing the activity of matrix-degrading enzymes in the area of acne lesions. This can be

accomplished by various means which are not mutually exclusive. One method is to disrupt the signalling caused by *P. acnes* byproducts that results in cytokines and MMPs. Another method is to disrupt the signalling that results in the recruitment of neutrophils with the accompanying neutrophil elastase and collagenase. Our present results suggest the mechanism shown in Fig. 6, a cartoon depicting the signalling in acne lesions. The *P. acnes* products induce keratinocytes (KC) to produce tumor necrosis factor alpha (TNF $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), and interleukins-8 and 10 (IL-8, IL-10), all cytokines. These cytokines induce resident skin cells to produce MMPs which degrade the dermal matrix. They also cause inflammation (e.g., redness, vasodilation, etc.) which is a signal for recruitment of neutrophils containing collagenase and elastase to the acne lesion; the neutrophil collagenase and the elastase contribute to degradation of the dermal matrix. The lack of procollagen biosynthesis, as shown in Fig. 4B, contributes to imperfect repair of the matrix. The end result is scarring.

To thwart the apparently inevitable result of acne scarring and inflammation, this invention disrupts the signalling pathways. More particularly, this invention uses an non-retinoid, non-steriod topically-applied composition, optionally in combination with a retinoid and/or a steriod, to inhibit this signalling, whereby degradation of the matrix is decreased and procollagen biosynthesis is restored, allowing the skin to heal with less scarring and less inflammation. Aspirin and E5510 (described by Fujimori, T., et al., Jpn J Pharmacol (1991) 55(l):81-91) inhibit NF- $\kappa$ B activation. Farnesyl transferase inhibitors such as B-581 (described by Garcia A.M., et al., J Biol Chem (1993) 268(25):18415-18), BZA-5B (described by Dalton M.B. et al., Cancer Res (1995) 55(15):3295-3304), farnesyl acetate, and ( $\alpha$ -hydroxyfarnesyl) phosphoric acid act on RAS and thus inhibit activation of the resultant ERK cascade; ERK leads to c-fos, which heterodimerizes with c-jun to create AP-1. Other useful inhibitors are those that inhibit NF- $\kappa$ B, such as sulfasalazine and parthenolide, serine protease (elastase) inhibitors, and antiadhesion molecules such as neutrophil infiltration inhibitors (e.g., selectin antagonists). As described in the aforementioned applications relating to UV-induction of MMPs, we have shown that so-called "antioxidants", like N-acetylcysteine (NAC), are useful at inhibiting MMPs, and have been shown in the literature (discussed below) to inhibit AP-1, NF- $\kappa$ B, and IL-8. Because the signalling that we have identified (which contributes to scarring and

inflammation in acne) appears similar to the signalling by which UV irradiation induces MMPs, similar "antioxidants" as disclosed in those application and discussed below (e.g., NAC, FDO) are likely to be useful for combatting acne scarring and inflammation.

As used herein, "inhibitors" of MMPs and other dermal matrix-degrading enzymes, such as elastase, inhibit one or more of the steps in the natural physiological pathways leading to the production of these enzymes and/or directly inhibit one or more of these proteases, or they directly inhibit the activity of the enzyme. Thus, as used herein an "inhibitor" excludes retinoids, inasmuch as retinoids and tetracyclines have been known for treating acne, this invention is directed to the novel use of a non-retinoid enzyme inhibitor, which use may be combined with the conventional use of a retinoid and/or a tetracycline. Thus, an "inhibitor" is a non-retinoid compound that directly inhibits one or more dermal matrix-degrading enzymes and/or indirectly inhibits the enzyme by inhibiting some portion of an upstream pathway(s) leading to one or more of these dermal matrix-degrading enzymes. Inhibition of the upstream pathway of these dermal matrix-degrading enzymes includes inhibition of one or more of the various signalling compounds and/or of the transcription factors (e.g., NF- $\kappa$ B, or cJUN and cFOS which together create AP-1) by which these enzymes are produced naturally.

MMPs are also inhibited by BB2284 (described by Gearing, A.J.H. et al., *Nature* (1994) 370:555-557), GI129471 (described by McGeehan G.M., et al., *Nature* (1994) 370:558-561), and TIMPs (tissue inhibitors of metalloproteinases, which inhibit vertebrate collagenases and other metalloproteases, including gelatinase and stromelysin). Other compounds useful for the present invention are direct MMP inhibitors such as hydroxamate and hydroxy-urea derivatives, the latter exemplified by Galardin, Batimastat, and Marimastat, and those disclosed in EP-A1-0 558635 and EP-A1-0 558648 (disclosed as useful for inhibiting MMPs in the treatment of, among other etiologies, skin ulcers, skin cancer, and epidermolysis bullosa).

Indirect MMP inhibitors include kinase inhibitors genistein and quercetin (as described in US 5637703, US 5665367, and FR-A-2,671,724, the disclosures of which are incorporated herein by reference) and related compounds, as well as other antioxidants such as NAC (N-acetyl cysteine), discussed below. Still further, other

kinase inhibitors are SB202190 (described by Lee, J.C., et al., Nature (1994) 372:739-746) and PD98059 (described by Dudley, D.T., et al., PNAS (USA) (1995) 92:7686-7689) inhibit specific kinases in the cascades, geranyl geranyltransferase inhibitors and lisofylline, which inhibit activation of the JNK cascade resulting from RAC/CDC42 activation, and U0126 (1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio]butadiene). As noted above, compounds that inhibit cytokines are indirect MMP inhibitors because interrupting the signalling pathway effectively inhibits MMPs. Compounds such as

As mentioned, various compounds termed "antioxidants" are also useful as MMP inhibitors. While not desirous of being constrained to any particular theory of operation, these compounds may quench or otherwise reduce free radicals and reactive oxygen species which may initiate or lead to MMP induction, such as via the MAP kinase cascade. These compounds include glutathione and its precursors, such as N-acetyl cysteine (NAC) or glutathione ethyl ester, more broadly N-CH<sub>3</sub>(CH<sub>2</sub>)<sub>n</sub>CO cysteine (wherein n is an integer from zero to eight, more preferably not more than 4), and related compounds and derivatives thereof as described in U.S. Pat. No. 5,296,500 (the disclosure of which is incorporated herein by reference). These other MMP inhibitors include water-soluble compounds such as vitamin C and NAC, and FDO. Various other compounds that may act as MMP inhibitors include: lipid-soluble compounds such as β-carotene and its derivatives or other carotenoids; glutathione and derivatives thereof (or of NAC); α-lipoic acid (1,2-dithiolane-3-pentanoic acid); selenium compounds such as Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one); isoflavones such as genistein (isoflavone), quercetin (flavon-3-ol), and pycnogenol (flavan-3-ol(s)); ergothioneine; saponin (e.g., from *Polypodium leucotomos*); ginkgo biloba extract (flavoneglycoside and terpenelactone) and feverfew (*Chrysanthemum parthenium*) extract (sesquiterpene lactone).

Inhibitors of activator protein-1 (AP-1) are likely to inhibit the subsequent signalling that results in the presence of MMPs in the dermal matrix; the more of the pathway that is inhibited, the more likely there will be no induction of MMPs. Among various compounds that have been found to inhibit AP-1 and may likely be used topically include the following. Cannabinoids: Faubert and Kaminski; "AP-1 activity is negatively regulated by cannabinol through inhibition of its protein components,

c-fos and c-jun", *J Leukoc Biol*, vol. 67, no. 2 (2000 Feb, pp. 259-66) (Cannabinoid compounds exhibit immunosuppressive actions that are putatively mediated through Gi-protein coupled receptors that negatively regulate adenylate cyclase. However, recent studies suggest that cannabinoids modulate other signaling cascades.

5 Cannabinol inhibited binding to AP-1-containing sites from the interleukin-2 promoter, in part, due to decreased nuclear expression of c-fos and c-jun. Thus, cannabinoid-induced immunosuppression involves disruption of the ERK signaling cascade.) Deferoxamine (DFO); Kramer-Stickland *et al.*, "Inhibitory effects of deferoxamine on UVB-induced AP-1 transactivation", *Carcinogenesis*, vol. 20, no. 11, Nov. 1999, pp. 2137-42 (Production of reactive oxygen species (ROS) by iron can contribute directly to DNA and protein damage and may contribute to cell signaling and proliferation. DFO treatment 24 h prior to UVB irradiation reduced UVB-induced AP-1 transactivation by approximately 80%, with the effect of DFO diminishing as pre-treatment time was shortened. Treatment with FeCl(3) a

10 11, Nov. 1999, pp. 2137-42 (Production of reactive oxygen species (ROS) by iron can contribute directly to DNA and protein damage and may contribute to cell signaling and proliferation. DFO treatment 24 h prior to UVB irradiation reduced UVB-induced AP-1 transactivation by approximately 80%, with the effect of DFO diminishing as pre-treatment time was shortened. Treatment with FeCl(3) a minimum of 6 h prior to UVB potentiated the UVB induction of AP-1 transactivation by 2-3-fold.) Separately, gadolinium chloride and alpha-tocopherol: Camandola *et al.*, "Liver AP-1 activation due to carbon tetrachloride is potentiated by 1,2-dibromoethane but is inhibited by alpha-tocopherol or gadolinium chloride", *Free Radic Biol Med*, vol. 26, no. 9-10, May 1999, pp. 1108-16. Cyclosporin A: Sugano *et al.*, "Cyclosporin A inhibits collagenase gene expression via AP-1 and JNK suppression in human gingival fibroblasts, *J Periodontal Res*, vol. 33, no. 8, Nov. 1998, pp. 448-452 (Cyclosporin A is able to affect signal transduction of lipidpolysaccharide-induced collagenase expression in fibroblasts; treatment of fibroblasts with LPS caused activation of collagenase gene, activator protein-1 (AP-1) and c-Jun N-terminal kinase (JNK). These activations were blocked by CsA. They suggest that inhibitory effects of CsA on LPS-induced signal transduction may contribute to the mechanism of CsA-induced gingival overgrowth. Catamins: Barthelman *et al.*, "(-)-Epigallocatechin-3-gallate inhibition of ultraviolet B-induced AP-1 activity", *Carcinogenesis*, vol. 19, no. 12, Dec. 1998, pp. 2201-4 (using cultured human keratinocytes, UVB-induced AP-1 activity is inhibited by EGCG in a dose range of 5.45 nM to 54.5 microM; EGCG is effective at inhibiting AP-1 activity when applied before, after or both before and after UVB irradiation; EGCG also inhibits AP-1 activity in the epidermis of a transgenic mouse model). Naphthopyranomycins

15 20 25 30

and exfoliamycins, such as K1115 A (Naruse *et al.*, "K1115A, , a new anthraquinone that inhibits the binding of activator protein-1 (AP-1) to its recognition sites. II. Taxonomy, fermentation, isolation, physico-chemical properties and structure determination," *J Antibiot* (Tokyo), vol 51, no. 6, June 1998, pp. 545-52; the anthraquinone 3,8-dihydroxy-1-propylantraquinone-2-carboxylic acid). DHEA: Dashtaki *et al.*, "Dehydroepiandrosterone and analogs inhibit DNA binding of AP-1 and airway smooth muscle proliferation", *J Pharmacol Exp Ther*, vol. 285, no. 2, 1998 May (pp. 876-83) (dehydroepiandrosterone (DHEA) and its analogs such as 16-alpha-bromoepiandrosterone). Oleanolic acid glycosides: Lee *et al.*, "Momordins inhibit both AP-1 function and cell proliferation," *Anticancer Res*, vol. 18, no. 1A, Jan-Feb 1999 (pp. 119-24). Monoterpene perillyl alcohol: Barthelman *et al.*, "Inhibitory effects of perillyl alcohol on UVB-induced murine skin cancer and AP-1 transactivation", *Cancer Res.*, vol. 58, no. 4, 15 Feb. 1998 (pp. 711-6). Curcumin, which inhibits both AP-1 and NF- $\kappa$ B: Xu *et al.*, "Curcumin inhibits IL1 alpha and TNF-alpha induction of AP-1 and NF- $\kappa$ B DNA-binding activity in bone marrow stromal cells," *Hematopathol Mol Hematol*, vol. 11, no. 1, 1997-8 (pp. 49-62); and Pendurthi *et al.*, "Suppression of activation of transcription factors Egr-1, AP-1 , and NF-kappa B," *Arterioscler Thromb Vasc Biol*, vol. 17, no. 12, Dec. 1997 (pp. 3406-13); and Bierhaus *et al.*, "The dietary pigment curcumin reduces endothelial tissue factor gene expression by inhibiting binding of AP-1 to the DNA and activation of NF-kappa B," *Thromb Haemost*, vol. 77, no. 4, 1997 April (pp. 772-82). Aspirin (acetylsalicylic acid): Huang *et al.*, "Inhibition of ultraviolet B-induced activator protein-1 (AP-1) activity by aspirin in AP-1-luciferase transgenic mice", *J Biol Chem*, vol. 272, no. 42, 17 Oct. 1997 (pp. 26325-31). Pyrrolidine dithiocarbamate and N-acetyl cysteine (inhibit AP-1, NF- $\kappa$ B, and IL-8): Munoz *et al.*, "Pyrrolidine dithiocarbamate inhibits the production of interleukin-6, interleukin-8, and granulocyte-macrophage colony-stimulating factor by human endothelial cells in response to inflammatory mediators: modulation of NF-kappa B and AP-1 transcription factors activity", *Blood*, vol. 88, no. 9, 1996 Nov 1 (pp. 3482-90). Metal salts, such as gold (I) and selenite: Handel *et al.*, "Inhibition of AP-1 binding and transcription by gold and selenium involving conserved cysteine residues in Jun and Fos," *Proc Natl Acad Sci USA*, vol. 92, no. 10, 1995 May 9 (pp. 4497-501) (in electrophoretic mobility-shift analyses, AP-1 DNA binding was inhibited by gold(I)

thiolates and selenite, with 50% inhibition occurring at approximately 5 microM and 1 microM, respectively; and other metal ions inhibited at higher concentrations, in a rank order correlating with their thiol binding affinities); and Spyrou *et al.*, "AP-1 DNA-binding activity is inhibited by selenite and selenodiglutathione", *FEBS Lett*, vol. 368, no. 1, 1995 Jul 10 (pp. 59-63) (selenite and selenodiglutathione (GS-Se-SG)); and Williams *et al.*, "Aurothioglucose inhibits induced NF-kB and AP-1 activity by acting as an IL-1 functional antagonist", *Biochim Biophys Acta*, vol. 1180, no. 1, 1992 Oct 13 (pp. 9-14).

Elastase inhibitors include procyanidines and proanthocyanidines, which non-competitively inhibit the activities of the proteolytic enzymes collagenase ( $IC_{50} = 38 \text{ nmol/l}$ ) and elastase ( $IC_{50} = 4.24 \text{ nmol/l}$ ) (*Arzneimittelforschung* (GERMANY) May 1994, 44 (5) p592-601), N-acetyl cysteine (e.g., US 5,637,616 for a disclosure of NAC as an inhibitor of proteases that result in mucosal or skin lesions) and derivatives thereof (as described in our copending application 89,914, filed 3 June 1998 (the disclosure of which is incorporated herein by reference)). Additional elastase inhibitors are described in the following disclosures. 6-Acylamino-2-(alkylsulfonyl)oxy-1H-isoindole-1,3-dione and related diones: Kerrigan *et al.*, "6-Acylamino-2-(alkylsulfonyl)oxy-1H-isoindole-1,3-dione mechanism-based inhibitors of human leukocyte elastase", *Bioorg Med Chem Lett*, vol. 10, no. 1, 2000 Jan 3 (pp. 27-30) (acylamino substitution in the 6-position increases selectivity and potency of these inhibitors for human leukocyte elastase); Gutschow *et al.*, "2-(diethylamino)thieno-1,3-oxazin-4-ones as stable inhibitors of human leukocyte elastase", *J Med Chem*, vol. 42, no. 26, 1999 Dec 30 (pp. 5437-47) (2-(diethylamino)thieno[1,3]oxazin-4-one). Caffeic acid derivatives: Melzig *et al.*, "Inhibition of granulocyte elastase activity by caffeic acid derivatives", *Pharmazie*, vol. 54, no. 9, 1999 Sep (pp. 712). Pyridyl esters of benzopyrans: Doucet *et al.*, "6-Substituted 2-oxo-2H-1-benzopyran-3-carboxylic acid as a core structure for specific inhibitors of human leukocyte elastase", *J Med Chem*, vol. 42, no. 20, 1999 Oct 7 (pp. 4161-71). Certain beta-lactams: Taylor *et al.*, "Novel mechanism of inhibition of elastase by beta-lactams is defined by two inhibitor crystal complexes", *J Biol Chem*, vol. 274, no. 35, 1999 Aug 27 (pp. 24901-5) (the presence of a hydroxyethyl substituent on the beta-lactam ring provides a new reaction pathway resulting in the elimination of the hydroxyethyl group and the formation of a



stabilizing conjugated double bond system); Wilmouth *et al.*, "Inhibition of elastase by N-sulfonylaryl beta-lactams: anatomy of a stable acyl-enzyme complex", *Biochemistry*, vol. 37, no. 50, 1998 Dec 15 (pp. 17506-13); pyrrolidone trans-lactams and trans-lactones) such as disclosed by Macdonald *et al.*, "Syntheses of trans-5-oxo-hexahydro-pyrrolo 3,2-bpyrroles and trans-5-oxo-hexahydro-furo 3,2-bpyrroles (pyrrolidine trans-lactams and trans-lactones): new pharmacophores for elastase inhibition", *J Med Chem*, vol. 41, no. 21, 1998 Oct 8 (pp. 3919-22). Benzoyl aminoacetic acid derivatives: Sakuma *et al.*, "ONO-5046 is a potent inhibitor of neutrophil elastase in human pleural effusion after lobectomy", *Eur J Pharmacol*, vol. 353, no. 2-3, 1998 Jul 24 (pp. 273-9) (sodium N- 2-4-(2,2-Dimethylpropionyloxy)phenyl-sulfonylamino-benzoyl-aminoacetic acid). Complex sulfates: Fujie *et al.*, "Release of neutrophil elastase and its role in tissue injury in acute inflammation: effect of the elastase inhibitor, FR134043", *Eur J Pharmacol*, vol. 374, no. 1, 1999 Jun 11 (pp. 117-25) (disodium-(Z,1S,15S,18S,24S,27R,29S,34S,37R)-29-benzyl-21-ethylidene-27-hydroxy-15-isobutyrylamino-34-isopropyl-31, 37-dimethyl-10,16,19,22,30,32,35,38-octaoxo-36-oxa-9,11,17,20,23,28,31,33-octaazatetracyclo 16,13,6,1(24,28)0(3,8)-octatricula-3,5,7-trien-5,6-diyl disulfate. Azaisochromens: Mitsuhashi *et al.*, "Pharmacological activities of TEI-8362, a novel inhibitor of human neutrophil elastase", *Br J Pharmacol*, vol. 126, no. 5, 1999 Mar (pp. 1147-52) (4-(N-(3-((3-carboxypropyl)amino)-8-methyl-1-oxo-4-azaisochromen-6-yl)carbamoyl)-4-((phenylmethoxy)carbonylamino)butanoic acid (C<sub>26</sub>H<sub>28</sub>N<sub>4</sub>O<sub>9</sub>)). Acetamides: Yamano *et al.*, "Protective effects of a PAF receptor antagonist and a neutrophil elastase inhibitor on multiple organ failure induced by cerulein plus lipopolysaccharide in rats", *Naunyn Schmiedebergs Arch Pharmacol*, vol. 358, no. 2, 1998 Aug (pp. 253-63) (2-(3-methylsulfonylamino-2-oxo-6-phenyl-1,2-dihydro-1-pyridyl)-N-(3,3,3-trifluoro-1-isopropyl-2-oxopropyl)acetamide). Molecules having only a few amino acid residues which are effective for penetrating the skin: Yamano *et al.*, "Protective effect of a pancreatic elastase inhibitor against a variety of acute pancreatitis in rats" *Jpn J Pharmacol*, vol. 77, no. 3, 1998 Jul (pp. 193-203) (trifluoroacetyl-L-lysyl-L-alaninanilide hydrochloride). Trifluoromethyl ketones: Huang *et al.*, "Effect of trifluoromethyl ketone-based elastase inhibitors on neutrophil function in vitro", *J Leukoc Biol*, vol. 64, no. 3, 1998 Sep (pp. 322-30) (new family of

elastase inhibitors ICI200355 and ZD0892). Sulfone derivatives of thiazolidine-3-ones: Groutas *et al.*, "Potent and specific inhibition of human leukocyte elastase, cathepsin G and proteinase 3 by sulfone derivatives employing the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold", *Bioorg Med Chem*, vol. 6, no. 6, 1998 Jun (pp. 661-71). Peptidyl trifluoromethylalcohols: Amour *et al.*, "Stereoselective synthesis of peptidyl trifluoromethyl alcohols and ketones: inhibitory potency against human leukocyte elastase, cathepsin G, porcine pancreatic elastase and HIV-1 protease", *J Pharm Pharmacol*, vol. 50, no. 6, 1998 Jun (pp. 593-600) (beta-peptidyl trifluoromethyl alcohols (TFMAs) Z-L-Val-NH-\*CH(Y)\*CH(OH)-CF<sub>3</sub>, where \*C is the chiral centre, varied in the nature of the substituent Y, a phenylethyl -(CH<sub>2</sub>)<sub>2</sub>-C<sub>6</sub>H<sub>5</sub> or an isopropyl -CH(CH<sub>3</sub>)<sub>2</sub> group; phenylethyl had IC<sub>50</sub> = 15 µM, whereas isopropyl had IC<sub>50</sub> = 200 µM). Benzoylamino acetates: Shinguh *et al.*, "Biochemical and pharmacological characterization of FK706, a novel elastase inhibitor", *Eur J Pharmacol*, vol. 337, no. 1, 1997 Oct 15 (pp. 63-71) (FK706, sodium 2-4-(S)-1-(S)-2-(RS)-3,3,3-trifluoro-1-isopropyl-2-oxopropyl-aminocarbonyl-pyrrolidin-1-yl-carbonyl-2-methylpropyl-aminocarbonyl-benzoylamino acetate, C<sub>26</sub>H<sub>32</sub>F<sub>3</sub>N<sub>4</sub>NaO<sub>7</sub>, a synthetic water-soluble inhibitor of human neutrophil elastase). Cephalosporin derivatives: Rees *et al.*, "Inhibition of neutrophil elastase in CF sputum by L-658,758", *J Pharmacol Exp Ther*, vol. 283, no. 3, 1997 Dec (pp. 1201-6); Buynak *et al.*, "7-alkylidenecephalosporin esters as inhibitors of human leukocyte elastase", *J Med Chem*, vol. 40, no. 21, 1997 Oct 10 (pp. 3423-33) (7-alkylidene, 7-haloalkylidene, and 7-cyanomethylidene benzhydryl ester 7-(cyanomethylidene)cephalosporin sulfone derivatives). Azabicyclic compounds and perhydroindoles: Portevin *et al.*, "Dual inhibition of human leukocyte elastase and lipid peroxidation: in vitro and in vivo activities of azabicyclo 2.2.2-octane and perhydroindole derivatives", *J Med Chem*, vol. 40, no. 12, 1997 Jun 6, (pp. 1906-18) (selective human leukocyte elastase (HLE) inhibitors of the Val-Pro-Val type in which the central proline residue was replaced by nonnatural amino acids Phi ((2S,3aS,7aS)-perhydroindole-2-carboxylic acid) and Abo((3S)-2-azabicyclo-2.2.2-octane-3-carboxylic acid). Trialkylammonium salts: Kouadri-Boudjelthia and Wallach, "Hydrophobic interactions are involved in the inhibition of human leukocyte elastase by alkyltrimethylammonium salts", *Int J Biochem Cell Biol*, vol. 29, no. 2, 1997 Feb (pp. 353-9) (preferably alkyl chain longer than ten carbon atoms).

Pivaloyloxy benzene derivatives: Imaki *et al.*, "Non-peptidic inhibitors of human neutrophil elastase : the design and synthesis of sulfonanilide-containing inhibitors", *Bioorg Med Chem*, vol. 4, no. 12, 1996 Dec. (pp. 2115-34) ( sulfonanilide-containing analogues most promising). Functionalized N-aryl azetidin-2-ones: Joyeau *et al.*,  
5 "Synthesis and inhibition of human leucocyte elastase by functionalized N-aryl azetidin-2-ones: effect of different substituents on the aromatic ring", *J Pharm Pharmacol*, vol. 48, no. 12, 1996 Dec. (pp. 1218-30)  
(N-aryl-3,3-difluoroazetidin-2-ones featured by a latent electrophilic methylene quinoniminium moiety, and incorporate on their aromatic ring either an alkyl moiety,  
10 a methoxy substituent or a carboxylic group; some proved to be good inactivators of human leucocyte elastase). Saccharine derivatives: Groutas *et al.*, "Design, synthesis, and in vitro inhibitory activity toward human leukocyte elastase, cathepsin G, and proteinase 3 of saccharin-derived sulfones and congeners", *Bioorg Med Chem*, vol. 4, no. 9, 1996 Sep. (pp. 1393-400) (derivatives has sulfinate leaving  
15 group; inhibitory activity is dependent on the nature and pKa of the leaving group, and the synthesized saccharin derivatives exhibit selective inhibition toward HLE). Mucopolysaccharides, such as heparin: Volpi, "Inhibition of human leukocyte elastase activity by heparins: influence of charge density", *Biochim Biophys Acta*, vol. 1290, no. 3, 1996 Aug 13 (pp. 299-307) (heparins strongly inhibit elastase  
20 activity, and there is a significant linear dependence between charge density (sulfate-to-carboxyl ratio) and enzymatic activity). Exopolysaccharides: Ying *et al.*, "Alginate, the slime exopolysaccharide of *Pseudomonas aeruginosa*, binds human leukocyte elastase, retards inhibition by alpha 1-proteinase inhibitor , and  
25 accelerates inhibition by secretory leukoprotease inhibitor", *Am J Respir Cell Mol Biol*, vol. 15, no. 2, 1996 Aug. (pp. 283-91) (data support a model in which each elastase molecule interacts with a total of 19 uronic acid units on the alginate, primarily through electrostatic forces).

NF- $\kappa$ B inhibitors include those disclosed in the following references.

Cyclopentenone prostaglandins: Rossi *et al.*, "Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase", *Nature*, vol. 403, no. 6765,  
30 2000 Jan 6 (pp. 103-8). Quercetin and staurosporine: Peet and Li, "IkappaB kinases alpha and beta show a random sequential kinetic mechanism and are inhibited by staurosporine and quercetin", *J Biol Chem*, vol. 274, no. 46, 1999 Nov

12 (pp. 32655-61) (but not the quercetin analogue Daidzein). Nepalolide A: Wang *et al.*, "Nepalolide A inhibits the expression of inducible nitric oxide synthase by modulating the degradation of I $\kappa$ B-alpha and I $\kappa$ B-beta in C6 glioma cells and rat primary astrocytes", *Br J Pharmacol*, vol. 128, no. 2, 1999 Sep. (pp. 345-56).

5 Turmeric (curcumin): Plummer *et al.*, "Inhibition of cyclo-oxygenase 2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF-kappaB activation via the NIK/IKK signalling complex", *Oncogene*, vol. 18, no. 44, 1999 Oct 28 (pp. 6013-20). Salicylates: Stevenson *et al.*, "Salicylic acid and aspirin inhibit the activity of RSK2 kinase and repress RSK2-dependent transcription of cyclic AMP

10 response element binding protein- and NF-kappa B-responsive genes", *J Immunol*, vol. 163, no. 10, 1999 Nov 15 (pp. 5608-16). Diterpenes: de las Heras *et al.*, "Inhibition of NOS-2 expression in macrophages through the inactivation of NF-kappaB by andalusol", *Br J Pharmacol*, vol. 128, no. 3, 1999 Oct (pp. 605-12) (andalusol, ent-6 $\alpha$ ,8 $\alpha$ ,18-trihydroxy-13(16),14-labdadiene, is a naturally occurring

15 diterpene, isolated from *Sideritis foetens* (Lamiaceae). N-substituted benzamides: Liberg *et al.*, "N-substituted benzamides inhibit NFkappaB activation and induce apoptosis by separate mechanisms", *Br J Cancer*, vol. 81, no. 6, 1999 Nov (pp. 981-8). While not preferred due to potential toxicity issues, arsenic: Estrov *et al.*, "Phenylarsine oxide blocks interleukin-1 $\beta$ -induced activation of the nuclear

20 transcription factor NF- $\kappa$ B, inhibits proliferation, and induces apoptosis of acute myelogenous leukemia cells", *Blood*, vol. 94, no. 8, 1999 Oct 15 (pp. 2844-53). Genistein: Tabary *et al.*, "Genistein inhibits constitutive and inducible NFkappaB activation and decreases IL-8 production by human cystic fibrosis bronchial gland cells", *Am J Pathol*, vol. 155, no. 2, 1999 Aug (pp. 473-81). Theophylline: Tomita *et al.*, "Functional assay of NF-kappaB translocation into nuclei by laser scanning

25 cytometry: inhibitory effect by dexamethasone or theophylline", *Naunyn Schmiedebergs Arch Pharmacol*, vol. 359, no. 4, 1999 Apr (pp. 249-55). Cepharanthine: a plant alkaloid (I) (Merck Index 11, 306, 1981), and described in U.S. Pat. Nos. 2,206,407 and 2,248,241, and Japanese Patents 120,483, 128,533,

30 and 141,292. Trifluoroalkyl salicylates: Bayon *et al.*, "4-trifluoromethyl derivatives of salicylate, triflusal and its main metabolite 2-hydroxy-4-trifluoromethylbenzoic acid, are potent inhibitors of nuclear factor kappaB activation", *Br J Pharmacol*, vol. 126, no. 6, 1999 Mar (pp. 1359-66) (2-hydroxy-4-trifluoromethylbenzoic acid (HTB) and

2-acetoxy-4-trifluoromethylbenzoic acid (triflusal), both more potent than aspirin or salicylate as inhibitors of NF- $\kappa$ B, indicating that the incorporation of a 4-trifluoromethyl group to the salicylate molecule strongly enhances its inhibitory effect on NF- $\kappa$ B activation). Quinapril: quinapril hydrochloride is chemically described as [3S-[2[R\*(R\*)],3R\*]]-2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid, monohydrochloride. Its empirical formula is C<sub>25</sub>H<sub>30</sub>N<sub>2</sub>O<sub>5</sub> •HCl . Cyclosporine A: Meyer *et al.*, "Cyclosporine A is an uncompetitive inhibitor of proteasome activity and prevents NF-kappaB activation", *FEBS Lett*, vol. 413, no. 2, 1997 Aug 18 (pp. 354-8). Arachidonic acid derivatives: Thommensen *et al.*, "Selective inhibitors of cytosolic or secretory phospholipase A2 block TNF-induced activation of transcription factor nuclear factor-kappa B and expression of ICAM-1", *J Immunol*, vol. 161, no. 7, 1998 Oct 1 (pp. 3421-30) (TNF-induced activation of NF- $\kappa$ B inhibited by trifluoromethyl ketone analogue of arachidonic acid (AACOCF<sub>3</sub>), methyl arachidonyl fluorophosphate, trifluoromethyl ketone analogue of eicosapentaenoic acid (EPACOCF<sub>3</sub>), 12-epi-scalaradial, and LY311727; arachidonyl methyl ketone analogue (AACOCH<sub>3</sub>) and the eicosapentanoyl analogue (EPACHOHCF<sub>3</sub>) had no effect on TNF-induced NF- $\kappa$ B activation. Genistein, erbstatin: Natarajan *et al.*, "Protein tyrosine kinase inhibitors block tumor necrosis factor-induced activation of nuclear factor- $\kappa$ B, degradation of I $\kappa$ B $\alpha$ , nuclear translocation of p65, and subsequent gene expression", *Arch Biochem Biophys*, vol. 352, no. 1, 1998 Apr 1 (pp. 59-70). Fasudil: 1-(5-isoquinolinesulfonyl)homopiperazine hydrochloride (fasudil hydrochloride); Sato *et al.*, "Inhibition of human immunodeficiency virus type 1 replication by a bioavailable serine/threonine kinase inhibitor , fasudil hydrochloride", *AIDS Res Hum Retroviruses*, vol. 14, no. 4 ,1998 Mar 1 (pp. 293-8). ACE (angiotensin converting enzyme) inhibitors, like quinipril: Hernandez-Presa *et al.*, "Angiotensin-converting enzyme inhibition prevents arterial nuclear factor-kappa B activation, monocyte chemoattractant protein-1 expression, and macrophage infiltration in a rabbit model of early accelerated atherosclerosis", *Circulation*, vol. 95, no. 6, 1997 Mar 18 (pp. 1532-41). Synthetic 1,3,7-trialkyl xanthine derivatives , such as pentoxifylline (3,7-dimethyl-1-(5-oxohexyl)xanthine; *Drugs & Aging* 1995, 7/6: 480-503) and denbufylline (1,3-dibutyl-7-(2-oxopropyl)xanthine); Lee *et al.*, "Pentoxifylline blocks hepatic stellate cell activation independently of phosphodiesterase inhibitory

activity", *Am J Physiol*, vol. 273, no. 5 Pt 1, 1997 Nov (pp. G1094-100).

Benzophenanthradine derivatives: Chaturvedi *et al.*, "Sanguinarine (pseudocheilerythrine) is a potent inhibitor of NF- $\kappa$ B activation, I $\kappa$ B $\alpha$  phosphorylation, and degradation", *J Biol Chem*, vol. 272, no. 48, 1997 Nov 28 (pp. 30129-34) (sanguinarine, a benzophenanthridine alkaloid). Actinomycin D: Faggioli *et al.*, "Protein synthesis inhibitors cycloheximide and anisomycin induce interleukin-6 gene expression and activate transcription factor NF- $\kappa$ B", *Biochem Biophys Res Commun*, vol. 233, no. 2, 1997 Apr 17 (pp. 507-13) (IL-6 mRNA accumulation in two human cell lines, MDA-MB-231 and HeLa, stimulated by cycloheximide or anisomycin is almost completely inhibited in the presence of actinomycin D). Hydroxyanthranilic acids: Sekkai *et al.*, "Inhibition of nitric oxide synthase expression and activity in macrophages by 3-hydroxyanthranilic acid, a tryptophan metabolite", *Arch Biochem Biophys*, vol. 340, no. 1, 1997 Apr 1 (pp. 117-23) (3-hydroxyanthranilic acid but not anthranilic acid). Nordihydroguaiaretic acid and AA861: Lee *et al.*, "Inhibition of 5-lipoxygenase blocks IL-1 beta-induced vascular adhesion molecule-1 gene expression in human endothelial cells", *J Immunol*, vol. 158, no. 7, 1997 Apr 1 (pp. 3401-7). Prostaglandin A1: Rossi *et al.*, "Inhibition of nuclear factor kappa B by prostaglandin A1: an effect associated with heat shock transcription factor activation", *Proc Natl Acad Sci USA*, vol. 94, no. 2, 1997 Jan 21 (pp. 746-50).

Sialyl Lewis X (SLe<sup>sup.x</sup>) mediates binding of neutrophils to vascular endothelial cells by binding to E-selectin. (M. Phillips, *et al.*, *Science* 1990, 250, 1130.; J. Lowe, *et al.*, *Cell* 1990, 63, 475; T. Feizi, *Trends Biochem Sci* 1991, 16, 84; M. Tiemeyer, *et al.*, *Proc. Natl. Acad. Sci. USA* 1991, 88, 1138; L. Lasky, *Science* 1992, 258, 964; and T. Springer, L. A. Lasky, *Nature* 1991, 349, 196.) Sialyl Lewis X (SLe<sup>sup.x</sup>) is a cell surface carbohydrate ligand found on neutrophils, anchored onto the outer membrane thereof by integral membrane glycoproteins and/or glycolipids. Administration of SLe<sup>sup.x</sup> inhibits the SLe<sup>sup.x</sup>/E-selectin interaction and blocks adhesion of neutrophils to endothelial cells. (M. Buerke, *et al.*, *J. Clin. Invest.*, 1994, 1140.). Neutrophil-mediated inflammatory diseases may be treated by administration of Sialyl Lewis X (SLe<sup>sup.x</sup>). Selectin inhibitor include those in the following references. E-, P-, and L-selectin inhibitors in US 5,830,871. Sulfatides and sialylated or sulfated fucooligosaccharides, as described in US 5,985,852, and other fucose derivatives as described in US 5,962,422 and US 5,919,769; as well as

described by Ikami *et al.*, "Synthetic studies on selectin ligands-inhibitors: Synthesis and inhibitory activity of 2-O-fucosyl sulfatides containing 2-branched fatty alkyl residues in place of ceramide", *Journal of Carbohydrate Chemistry*, vol. 17, no. 3, 1998 (pp. 453-470) (sulfated 2-O-alpha-L-fucopyranosyl beta-D-galactopyranosides containing 2-branched fatty-alkyl residues in place of ceramide); Todderud *et al.*, "BMS-190394, a selectin inhibitor, prevents rat cutaneous inflammatory reactions", *J Pharmacol Exp Ther*, vol. 282, no. 3, 1997 Sep (pp. 1298-304) (selectin antagonist BMS-190394, a structural analog of sulfatide). TBC-1269 (available from Texas Biotechnology Corp., Houston, TX) and other mannose derivatives: for example, Dupre *et al.*, "Glycomimetic selectin inhibitors: (alpha-D-mannopyranosyloxy)-methylbiphenyls", *Bioorganic & Medicinal Chemistry Letters*, vol. 6, no. 5, 1996 (pp. 569-572); Lin *et al.*, "Synthesis of sialyl Lewis x mimetics as selectin inhibitors by enzymatic aldol condensation reactions", *Bioorg Med Chem*, vol. 7, no. 3, 1999 Mar (pp. 425-33) (D-mannosyl phosphate/phosphonate derivatives enzymatically prepared as sialyl Lewis x tetrasaccharide mimics); Kogan *et al.*, "Rational design and synthesis of small molecule, non-oligosaccharide selectin inhibitors: (alpha-D-mannopyranosyloxy)biphenyl-substituted carboxylic acids", *J Med Chem*, vol. 38, no. 26, 1995 Dec 22 (pp. 4976-84). Leumedins: Endemann *et al.*, "Novel anti-inflammatory compounds induce shedding of L-selectin and block primary capture of neutrophils under flow conditions", *J Immunol* 1997 May 15; 158(10):4879-85 (leumedins are small molecules that inhibit neutrophil movement into inflamed tissues). Di- and tri-valent small molecules, mainly 3-carboxyaralkyl-substituted 2-alpha-D-mannopyranosyloxy-phenyl unsubstituted, oxygen-, or nitrogen-substituted alkanes (*e.g.*, oxobutane, piperidine), as described in US 5,919,768. GSC-150: Wada *et al.*, "Effect of GSC-150, a new synthetic selectin inhibitor, on skin inflammation in mice", *Japanese Journal of Pharmacology*, vol. 71, no. Suppl. 1, 1996 (Page 302P). Sialyl Lewis x analogs: Kiso *et al.*, "Studies of selectin binding inhibitors: Synthesis of sialyl-Lewis x and sialyl-Lewis a epitope analogs containing 2-acetamido derivative of N-methyl-1-deoxynojirimycin", *Journal of Carbohydrate Chemistry*, vol. 15, no. 1, 1996 (pp. 1-14) (synthesis of sialyl-Lewis x (15) and sialyl-Lewis a (17) epitope analogs containing the 2-acetamido derivative of N-methyl-1-deoxynojirimycin). Glycolipid sulfatide: Nair *et al.*, "Inhibition of immune complex-induced inflammation by a small molecular weight selectin antagonist",

*Mediators of Inflammation*, vol. 3, no. 6, 1994 (pp. 459-463). Triterpene glucosides such as glycyrrhizin: Rao *et al.*, "Glycyrrhetic acid glycosides are sialyl Lewis X mimics, and function as selectin inhibitors", *Molecular Biology of the Cell*, vol. 5, no. Suppl., 1994 (pp. 480A); Narasinga *et al.*, "Sialyl Lewis X Mimics Derived from a Pharmacophore Search Are Selectin Inhibitors with Anti-inflammatory Activity", *Journal of Biological Chemistry*, vol. 269, no. 31, 1994 (pp. 19663-19666) (glycyrrhizin, an L-fucose derivative, and a C-fucoside derivative; Subramanian *et al.*, "Attenuation of renal ischemia-reperfusion injury with selectin inhibition in a rabbit model", *Am J Surg*, vol. 178, no. 6, 1999 Dec (pp. 573-6). GM-1925: Cornell and Bowyer, "Attenuation of lung injury in a rabbit acid aspiration model using GM-1925, a novel selectin inhibitor", *Surgical Forum*, vol. 45, 1994 (pp. 107-110). Diisopropyl fluorophosphate: Palecanda *et al.*, "Complete inhibition of cross-linking and activation induced shedding of I selectin by the serine protease inhibitor diisopropyl fluorophosphate DPF", *J Immunol*, vol. 150, no. 8 Part 2, 1993 (page 304A). BR 44-09 and BR 44-096837: Heavner *et al.*, "Multiple binding site involvement in neutrophil selectin adhesion implications for design of peptide and carbohydrate inhibitors BIO BR 44-09 BR 44-096840", *J Cell Biochem Suppl*, no. 17 Part A, 1993 (p. 342); Dalton *et al.*, "Inhibition of selectin mediated adhesion in-vivo and in-vitro BIO BR 44-09 BR 44-096837", *J Cell Biochem Suppl*, no. 17 Part A, 1993 (p. 342). GMP-140: May *et al.*, "GMP-140 P Selectin inhibits human neutrophil activation by lipopolysaccharide analysis by proton magnetic resonance spectroscopy BIO BA 93-00 BA 93-130631", *Biochem Biophys Res Commun*, vol. 183, no. 3, 1992 (pp. 1062-1069). Tetrasaccharides: Ushakova *et al.*, "Inhibitory activity of monomeric and polymeric selectin ligands", *Vopr Med Khim*, vol. 45, no. 5, 1999 Sep-Oct (pp. 375-83) (tetrasaccharides SiaLex, SiaLea, HSO<sub>3</sub>Lex, their conjugates with polyacrylamide (40 kDa), and several other monomeric and polymeric substances; all monomeric inhibitors were about two orders of magnitude weaker; PAA-conjugates, containing as a ligand tyrosine-o-sulfate in addition to one of the above mentioned oligosaccharides, were the most potent synthetic blockers compared with fucoidan, bi-ligand glycoconjugate HSO<sub>3</sub>Lea-PAA-sTyr); Bertozzi *et al.*, "Sulfated disaccharide inhibitors of L-selectin: deriving structural leads from a physiological selectin ligand", *Biochemistry*, vol. 34, no. 44, 1995 Nov 7 (pp. 14271-8) (generated a simple small molecule (lactose 6',6-disulfate) with greater



inhibitory potency for L-selectin than sialyl Lewis x). Panosialins: Shinoda *et al.*, "Panosialins, inhibitors of an alpha1,3-fucosyltransferase Fuc-TVII, suppress the expression of selectin ligands on U937 cells", *Glycoconj J*, vol. 15, no. 11, 1998 Nov (pp. 1079-83). CY-1503: Schmid *et al.*, "Carbohydrate selectin inhibitor CY-1503 reduces neutrophil migration and reperfusion injury in canine pulmonary allografts", *J Heart Lung Transplant*, vol. 16, no. 10, 1997 Oct (pp. 1054-61).

Inhibitors of TLRs (toll-like receptors) and/or other receptors that are sensitive to the LPS-like compounds associated with acne lesions can be used to ameliorate the signalling that induces the cytokines  $\text{TNF}\alpha$ , IL-1 $\beta$ , IL-8, and IL-10, as shown in Figs. 6 and 8B, and any other related cytokines that are induced by the *P. acnes* bacteria. Diglucosamine-based LPS antagonists include E5564 and E5531, described by E. Lien *et al.*, *J. Biol. Chem.* 276(3): 1873-80 (2001), and by T.K. Means *et al.*, *J. Immunol.*, 166(6): 4074-82 (2001), inhibit certain TLRs.

Generally, molecules having a molecular weight of less than about 600 will pass through the skin, and lipophilic molecules are preferred (or a conjugate having a lipophilic portion). Accordingly, while short chain peptides are not listed above, those having a low molecular weight and a high proportion of lipophilic amino acid residues are likely to be useful as topical inhibitors of AP-1, NF- $\kappa$ B, elastase, and/or selectin.

Figs. 7A-7F show the effect of a control and some of these inhibitors on acne-affected skin. Each of these figures is an *in-situ* zymogram showing collagenase (MMP-1 and/or MMP-8) activity in a biopsied section; green is fluorescently-labelled collagen placed on a slide, over which is placed a biopsy section from an acne lesion from a human volunteer. Fig. 7A is a zymogram of a biopsy of uninvolved (not acne-affected) skin; there is almost no collagenase activity. Fig. 7B is a zymogram of acne-involved skin; there is significant collagenase activity as evidenced by the dark (black) areas where the fluorescently-labelled collagen has been degraded by the collagenase in the biopsied specimen. Fig. 7C is a zymogram of acne-involved skin which was treated with a control compound C1006 structurally analogous to known inhibitors but found to be inactive (the subject compound is applied over the biopsy section laid on the fluorescently-labelled collagen-coated slide); as seen by the dark areas, there was still significant collagen degradation (and hence collagenase activity). Fig. 7D is a zymogram of acne-affected skin treated with a

collagenase inhibitor AG 3340 (Drugs R D 1999 Feb;1(2):137-8); the amount of collagenase activity is minimal and comparable with that seen for uninvolved skin. Fig. 7E is also a zymogram of acne-involved skin treated with collagenase inhibitor GM1489; again there is significant inhibition of collagenase activity. Finally, Fig. 7F is a zymogram of acne-involved skin treated with GM6001; again there is significant suppression of collagenase activity. The MMP inhibitor GM6001 is N-[(2R)-2-hydroxamidocarbonylmethyl]-4-methylpentanoyl]-L-tryptophan methylamide (ilomastat) (see RE Galardy *et al.*, *Ann. NY Acad. Sci.*, 732:315-323 (1994)). The inhibitor GM1489 is N-[(2R)-2-(carboxymethyl)-4-methylpentanoyl]-L-tryptophan methylbenzylamide (see WM Holleran *et al.*, (1997) *Arch. Dermatol. Res.* 289:138-144). The control compound C1006 is N-*t*-Butyloxycarbonyl-L-leucyl-L-tryptophan methylamide. These three compounds (GM6001, GM1489, and C1006, were obtained from AMS Scientific Inc., Concord, California).

The compositions of this invention can be provided in any cosmetically suitable form, preferably as a lotion or cream, but also in an ointment or oil base, as well as a sprayable liquid form (e.g., a spray that includes the MMP inhibitor in a base, vehicle, or carrier that dries in a cosmetically acceptable way without the greasy appearance that a lotion or ointment would have if applied to the skin).

In addition, the compositions contemplated by this invention can include one or more compatible cosmetically acceptable adjuvants commonly used, such as colorants, fragrances, emollients, humectants, and the like, as well as botanicals such as aloe, chamolile, and the like.

When used topically, an inhibitor (of a dermal matrix-degrading enzyme) is used preferably at concentrations of between about 0.05% and about 5%, more preferably between 0.1% and 1%; antioxidants are preferably taken in "megadoses" (e.g., at least 1 g/d of vitamin C, at least 1000 I.U. of one or more tocopherols). A direct inhibitor includes AG3340, used at 0.3%  $\pm$  0.1%.

In view of the foregoing, another facet of this invention is the use of an MMP inhibitor in combination with a clinical therapy for acne. The various treatments for acne, as noted in the Background section, involve the topical or oral administration of any number of active ingredients, ranging from antibacterials to anti-inflammatories. By virtue of this invention, combination therapies, such as combined oral and topical administration of tetracycline (which may involve use of

two different tetracyclines), combined oral and topical administration of a retinoid, or a combination topical composition containing (i) an MMP inhibitor and/or an elastase inhibitor and (ii) another compound (such as an antibiotic, comedolytic, and/or anti-inflammatory).

5 In another aspect this invention includes an improved process for treating acne. As mentioned above, retinoids are known and presently used for treating acne. According to this invention, the improvement to that process is the use of a compound that inhibits the degradation of the retinoid. One enzyme that degrades retinoids and can be inhibited is cytochrome P-450. In the skin, retinoids are  
10 converted into retinoic acid (RA) as the active form. Natural retinoids that function in the skin are all *trans* or are metabolized to all *trans*. Retinoic acid (RA; all *trans*) is metabolized to inactivation by hydroxylation (via RA 4-hydroxylase) to 4-hydroxy-RA, which is then oxidized by a reaction mediated by the cytochrome P-450-dependent monooxygenase system. (S. Kang *et al.*, "Liarozole Inhibits Human Epidermal Retinoic Acid 4-Hydroxylase Activity and Differentially Augments Human Skin Responses to Retinoic Acid and Retinol *In Vivo*," *J. Invest. Dermatol.*, 107:183-187 (Aug. 1996); E.A. Duell *et al.*, "Human Skin Levels of Retinoic Acid and Cytochrome P-450-derived 4-Hydroxyretinoic Acid after Topical Application of Retinoic Acid *In Vivo* Compared to Concentrations Required to Stimulate Retinoic Acid Receptor-mediated Transcription *In Vitro*," *J. Clin. Invest., Skin Retinoid Levels and Reporter Gene Activity*, 90:1269-1274 (Oct. 1992); E.A. Deull *et al.*, "Retinoic Acid Isomers Applied to Human Skin *in Vivo* Each Induce a 4-Hydroxylase That Inactivates Only *Trans* Retinoic Acid," *J. Invest. Dermatol.*, 106:316-320 (Feb. 1996); the disclosures of which are incorporated herein by reference). Accordingly, compounds which  
25 interfere with the elimination metabolism of all *trans* RA, the active metabolite of topically applied retinoids such as 9-*cis* RA and 13-*cis* RA, will beneficially increase the amount of RA in the skin. Thus, preventing the degradation of natural (all *trans*) RA in the skin effectively increases its concentration, and so provides the benefits useful for its treatment of acne.

30 Retinoids that are or may likely be useful for treating acne include natural and synthetic analogs of vitamin A (retinol), vitamin A aldehyde (retinal), vitamin A acid (retinoic acid (RA)), including all-*trans*, 9-*cis*, and 13-*cis* retinoic acid), etretinate, and others as described in EP-A2-0 379367, US 4,887,805, and US 4,888,342 (the

disclosures of which are all incorporated herein by reference), and the dissociating retinoids that are specific for AP-1 antagonism (such as those described by Fanjul, et al. in Nature (1994) 372:104-110). Various synthetic retinoids and compounds having retinoid activity are expected to be useful in this invention, to the extent that they exhibit anti-MMP activity *in vivo*, and such are described in various patents assigned on their face to Allergan Inc., such as in the following U.S. Patents, numbered: 5,514,825; 5,698,700; 5,696,162; 5,688,957; 5,677,451; 5,677,323; 5,677,320; 5,675,033; 5,675,024; 5,672,710; 5,688,175; 5,663,367; 5,663,357; 5,663,347; 5,648,514; 5,648,503; 5,618,943; 5,618,931; 5,618,836; 5,605,915; 5,602,130. Still other compounds described as having retinoid activity are described in other U.S. Patents, numbered: 5,648,563; 5,648,385; 5,618,839; 5,559,248; 5,616,712; 5,616,597; 5,602,135; 5,599,819; 5,556,996; 5,534,516; 5,516,904; 5,498,755; 5,470,999; 5,468,879; 5,455,265; 5,451,605; 5,343,173; 5,426,118; 5,414,007; 5,407,937; 5,399,586; 5,399,561; 5,391,753; and the like, the disclosures of all of the foregoing and following patents and literature references hereby incorporated herein by reference.

Examples of compounds dermatologically acceptable and having or likely to have inhibitory effects on the P-450-mediated degradation of RA and other retinoids include azoles, especially triazoles, including, for example, ketoconazole (US 4,144,346 and 4,223,036), fluconazole (US 4,404,216), itraconazole (US 4,267,179), liarozole, irtemazole, and the like; compounds related to these that may also be useful include, for example, diazines such as flucytosine.

It would also be beneficial to use such cytochrome P-450 inhibitors in combination with a reduced amount of retinoid; the P-450 inhibitor decreases the metabolic elimination of the retinoid and so less retinoid is needed to achieve the same result. Still further, analytical methods are available for determining whether a given compound inhibits the degradation of RA by applying the compound and testing for changes in CRABP (cytoplasmic retinoic acid binding protein), which will have increased levels if the levels of RA are also increased by the topical application of the test compound.

It would also be useful to use inhibitors of CD-14, a pattern recognition protein, part of the innate immune response of humans, that binds to LPS-like substances and activates TLRs.

## Methods Used in the Examples

The references noted in this section are incorporated herein by reference.

Preparation of skin supernatants for biochemical analysis. Skin samples were ground by mortar and pestle under liquid nitrogen, and homogenized in a Dounce tissue grinder in buffer containing 10 mM Hepes, 1 mM EDTA, 5 mM EGTA, 10 mM MgCl<sub>2</sub>, 50 mM glycerophosphate, 5 mM NaVO<sub>4</sub>, 2 mM DTT, 0.5 mM PMSF, 10 µg/ml aprotinin, 10 µg/ml leupeptin, and 10 µg/ml pepstatin, and 0.5% NP-40. Homogenates were centrifuged at 14,000 g for 15 min., and supernatants were collected and used for biochemical determinations as described herein.

Immunohistology: Immunohistology of Type I pN collagen, MMP-1, and neutrophil elastase were performed as has been described by Griffiths, C.E.M., *et al.*, *N. Engl. J. Med.*, 329:530-535 (1993). Type I pN collagen was detected with mouse monoclonal IgG1 antibody (SP1.D8; available from Univ. of Iowa Dept. of Biological Sciences Developmental Studies Hybridoma Bank, Iowa City, IA) raised against the aminopropeptide region of human Type I procollagen (Foellmer, H.G., *et al.*, *Euro. J. Biochem.*, 134:183-189 (1983)). The MMP-1 antibody is available from Comicon (Temecula, California), and the neutrophil elastase antibody is available from DAKO (Carpinterina, California).

Western analysis of proteins. Immunoreactive proteins were visualized by enhanced chemiluminescence detection and quantified by laser densitometry, or by enhanced chemifluorescence detection and quantified by a Storm imager (Molecular Dynamics, Palo Alto, CA).

In-situ Zymography: performed as described by Fisher *et al.* in "Pathophysiology of premature skin aging induced by ultraviolet radiation," *New Engl. J. Med.*, vol. 337, pp. 1419-1428 (1997).

The foregoing description is meant to be illustrative and not limiting. Various changes, modifications, and additions may become apparent to the skilled artisan upon a perusal of this specification, and such are meant to be within the scope and spirit of the invention as defined by the claims.